HSB Project 1
Benzene ADME in Genetically Diverse Mouse Strains

Project Leader
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Background and Rationale
The Host Susceptibility Branch (HSB) has been charged to develop model systems that mirror the genetic diversity of human populations. Under a separate contract, the NTP has sequenced 15 mouse strains derived from 3 mouse subspecies from different parts of the world that are more genetically diverse than the human population. There are more than 8 million single nucleotide polymorphisms (SNPs) and copy number variants (CNV) distributed throughout the mouse genome (Frazer *et al.*, 2007; Yang *et al.*, 2007). We aim to determine the use of multiple inbred strains with significant genetic diversity for correlation with chemical specific inbred strain and human metabolism data. To determine the utility of this approach, we are quantifying absorption, distribution, metabolism, and elimination (ADME) kinetics and toxicity relationships of model agents and NTP nominated chemicals. These data may be used to increase the potential for rodent to human extrapolation and risk characterization. Benzene was selected as an initial test agent based upon extensive studies in rodent and humans (Aksoy, 1989; Huff, 2007; Huff *et al.*, 1989; Lan *et al.*, 2004). Benzene is a ubiquitous environmental and occupational hematotoxicant and carcinogen. Metabolism of benzene is complex, requiring extensive phase 1 and 2 enzymes that produce a number of reactive intermediates with different mechanisms of toxicity (Qu *et al.*, 2003; Rappaport *et al.*, 2005; Recio *et al.*, 2005; Ross, 1996). After a century of research, the mechanisms of benzene toxicity are still poorly understood. ADME kinetics defines the bioavailability and elimination based upon the rates of absorption and elimination of parent compound and metabolites. Heritable variations in SNP and CNV are observed in both Phase 1 and phase 2 enzymes, but the heritable determinants that modify bioavailability that affect toxicity are unknown or poorly understood (Kim *et al.*, 2007; Lan *et al.*, 2009; Shen *et al.*, 2006; Smith *et al.*, 2004). By determining and understanding the genetic determinants of benzene ADME kinetics, we aim to increase our knowledge of individual responses to benzene exposure and toxicity.

Hypothesis
Genetic variation inherent in multiple inbred mouse strains will show significant differences in ADME phenotypes that determine bioavailability and toxicity.

Key Issues
Selection of rodent models to simulate the wide range of genetic and epigenetic diversity in the human population is critical to this research. We will investigate 18 genetically diverse inbred strains. Additional strains will be added as necessary to define the variable range of ADME response in multiple strains and to increase our understanding of strain differences. Statistical analysis will aid determination of differences in ADME kinetics between strains and aid identification of strains that share

haplotypes or strain dependent polymorphisms for genetic analysis. Identification of highly penetrant genes that modify these traits will aid intense bioinformatic analysis of heritable haplotypes or strain dependent polymorphisms that may lead to functional validation and define the range of genetic determinants that are critical to inclusion of genetics into hazard identification and risk characterization.

Specific Aims

Aim 1: Characterize the blood concentration curve and tissue distribution of benzene in C57BL/6J mice (male and female).

Aim 2: Characterize the AUC, C_{max}, and T_{max} of [^{14}C] radioactivity in blood following oral administration of [^{14}C] benzene to 17 genetically diverse strains of mice (male and female).

Aim 3: Identify benzene metabolites generated in 4 strain/gender combinations of mice that display a notable difference in C_{max} and/or T_{max}, as defined in Aim 2.

Aim 4: Characterize the dose-dependency of routes of elimination for benzene in the 4-strain/gender combinations of mice identified in Aims 2 and 3.

Aim 5: Characterize the expression and function of hepatic enzymes that are known to metabolize benzene in the 4-strain/gender combinations of mice identified in Aims 2, 3 and 4.

Approach

Characterize the blood pharmacokinetics and tissue distribution of [14C] benzene equivalents in blood following oral administration of [14C] benzene to 18 genetically diverse strains of mice (male and female) or more as required. Results will identify strain/gender combinations that display notable differences from male C57BL/6J mice as well as strains that show high versus low values in the parameters measured. Characterize the Cmax and Tmax of [14C] radioactivity in bone marrow following oral administration of [14C] benzene to 17 genetically diverse strains of mice (male and female). Identify benzene metabolites generated in 4 strain/gender combinations of mice that display a notable difference in Cmax and/or Tmax. Characterize the expression and function of hepatic enzymes that are known to metabolize benzene in the 4-strain/gender combinations of mice identified above. We will perform statistical analysis to aid the determination of differences between strains and to aid evaluation by haplotype association mapping to identify allelic variants affecting ADME kinetic parameters in order to understand differences in strain dependent ADME kinetics as described in the HSB benzene haplotype association mapping project.

Significance and Expected Outcome

The goal of this research is to model the range of genetic diversity in human populations that result in large differences in ADME constants, resulting in variable internal doses and bioavailability following chemical exposures. To accomplish this goal, studies are being conducted to characterize the effects of genetic variability on the ADME properties of [14C] benzene in 18 genetically diverse strains of mice. Currently the criteria to characterize strain differences are area under the curve (AUC) for total [14C] benzene-equivalents, maximum concentration of [14C] radioactivity reached in whole blood following oral administration of [14C] benzene (Cmax) and time to reach Cmax

(Tmax) following oral administration of [14C] benzene. To date, studies have been conducted in males or females of 18 strains: C57BL/6J, C3H/HeJ, B6C3F1J, BALB/cByJ, 129S1/SvImJ, A/J, AKR/J, FVB/NJ, BTBRT+FJ, DBA/2J, KK/HiJ, NOD/ShiLtJ, NZW/LacJ, PWD/PhJ, PWK/EiJ, CAST/EiJ, MOLF/EiJ and WSB/EiJ.

The results of these strains show enormous variation, with apparent Cmax values varied approximately 7-fold, ranging from 226-1580 nmol-eq/mL and Tmax values ranging from less than 5 to 17.5 min. Apparent AUC values varied greater than 10-fold, ranging from 23 to 239 µmol*min/mL. Values for the reference strain (C57BL/6J) were 359 nmol-eq/mL at 17 min with an AUC of 45 µmol*min/mL. Pharmacokinetic values represent a mixture of parent benzene and metabolites circulating systemically. Based on the 2-fold difference selection protocol for Cmax, Tmax and AUC values to date, males from the following strains have been selected for further study include: NZW/LacJ (high AUC, high Cmax), KK/HiJ (high Cmax), A/J (low Tmax), KK/HiJ (low Tmax), and NOD/ShiLt (low Tmax). Preliminary studies of total [14C] radioactivity disposition in bone marrow from femurs show a similar Tmax for [14C] radioactivity in bone marrow as [14C] radioactivity in systemic blood, albeit at lower levels. Statistical analysis of this data has confirmed significant differences between these 18 stains confirming the selections of strains for in depth investigation for tissue metabolite distribution.

Current and Future Activities

At present, the Benzene ADME studies conducted in females of these strains are undergoing final evaluation and quality control for in depth statistical analysis as described above. If required to improve power for haplotype mapping association and statistical analysis, additional strains of inbred mice will be investigated as described in both male and female mice in this study.

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